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| <p>2000-475857/41 B04 D16 J04 SIPP/ 1998.12.30 SIPPEL A E *WO 200040969-A1 1998.12.30 1998-1060833(+1998DE-1060833) (2000.07.13) G01N 33/566, C12N 5/10, C12Q 1/02</p> <p>Cell having conditionally activable Ras-dependent signaling pathway, useful for identifying receptor ligands that are potential therapeutic agents (Ger)</p> <p>C2000-142690 N(AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW) R(AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW)</p> <p>Addnl. Data: SIPPEL A E, ZIMMERMANN A ZIMMERMANN A (ZIMM/) 1999.12.29 1999WO-EP10460</p> | <p>B(3-L, 4-C1, 4-F1E, 4-H7, 4-N2, 4-N4, 6-A3, 11-C8E, 12-K4E, 14-S3) D(5-H9, 5-H12A, 5-H14, 5-H17A) J(4-B1B) .10</p> |
| <p>NOVELTY</p> <p>A cell (A) comprising a membrane receptor (MR), consisting of a ligand-binding domain (LBD), a membrane-localization signal (MLS) and a mediator domain (MD), is new.</p> | <p>DETAILED DESCRIPTION</p> <p>A cell (A) comprising a membrane receptor (MR), consisting of a ligand-binding domain (LBD), a membrane-localization signal (MLS) and a mediator domain (MD), is new.</p> <p>When a ligand binds to LBD, or in the absence of ligand binding to LBD, a structural alteration that affects MD occurs. This alteration allows an effector protein or polypeptide (EP), able to activate a Ras (or Ras-like) signaling pathway, to bind to a component of the membrane, optionally via an additional protein or polypeptide (adapter).</p> <p>INDEPENDENT CLAIMS are also included for the following:</p> <ol style="list-style-type: none"> (1) an <i>in vivo</i> assay for identifying or detecting ligands (L) of LBD using (A); (2) a screening method for unknown (L) of a specific receptor using the assay of (1); (3) an <i>in vivo</i> assay for quantitative determination of the concentration of (L) in a sample using (A); (4) an <i>in vivo</i> method for determining if a compound (I) can alter the <p>WO 200040969-A+</p> |

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| <p>binding activity of LBD of a particular receptor, using (A);</p> <ol style="list-style-type: none"> (5) an <i>in vivo</i> assay for determining if a polypeptide or protein (II) has the ligand-binding function of a receptor, using (A); (6) kits for any of the above assays; (7) (L), (I) and (II) produced or identified by the new methods; (8) a method for identifying polypeptides or proteins (IIa), especially receptors, having the ligand-binding function of a receptor, using (A); (9) a method for producing compounds by (multiple) derivation of compounds identified by the new methods; and (10) a nucleic acid (III) encoding (poly)peptides, especially receptors, identified or produced by these methods. <p>USE</p> <p>(A) are used to identify, detect and quantify ligands for LBD, compounds that alter binding of LBD to a particular ligand or proteins and polypeptides that have ligand-binding properties of a receptor. (L), (I) and (II) are potentially useful as therapeutic agents, or for development of such agents. Nucleic acids that encode (L), (I) and (II) are useful in gene therapy.</p> <p>ADVANTAGE</p> | <p>(A), particularly easily manipulated prokaryotic or yeast cells, allow rapid determination of ligand-receptor interactions.</p> <p>EXAMPLE</p> <p><i>Saccharomyces cerevisiae</i> cdc25-2 cells (having a mutant guanine nucleotide exchange factor that is inactive at about 36 °C) were transformed with:</p> <ol style="list-style-type: none"> (1) a vector providing constitutive expression of the AT1A angiotensin receptor of the rat; (2) one or more vectors that contain the rat G protein subunits alpha-13, beta-1 and gamma-3; and (3) an inducible vector expressing, in presence of galactose, a fusion protein of phosphatidylcholine phospholipase C without the membrane localization domain and the human Ras-protein fragment Ha-ras L61, lacking the CAAX box required for membrane localization. <p>The cells were incubated for 2-3 days at 37 °C in galactose-containing medium and in presence or absence of various additives. Cells which could grow only in presence of angiotensin or in the presence of the non-protein angiotensin receptor ligand L-162313 showed functionally active ligand receptor interaction.</p> <p>WO 200040969-A+/1</p> |
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| <p>2000-475857/41</p> <p>TECHNOLOGY FOCUS</p> <p>Biology - Preferred materials: EP is a guanine nucleotide exchange factor (GEF) or an active Ras-family protein, particularly the CDC25 protein of <i>Saccharomyces cerevisiae</i>, an SOS protein from a mammal or an SOS-like protein from other organisms, or their derivatives. Optionally EP is a fusion protein (FP) with an adapter protein that allows binding, optionally via additional adapters, to a membrane component. FP may require enzymatic modification before it can bind, and the activity required is provided only in absence or presence of ligand binding. The receptors are transmembrane, enzyme-coupled, G-protein coupled, seven-transmembrane or olfactory receptors, or they are synthetic receptors that contain the LBD of a natural receptor, i.e. any of those above or a nuclear receptor. In the synthetic receptors, LBD is altered by mutation or has been derived by molecular modeling. MD is particularly the cytoplasmic domain of a G-coupled receptor, or its functional derivative, and in this case FP can interact with MD following dissociation of the heterotrimeric G protein. Specifically FP is then derived from an active ras protein and a GRK 2</p> | <p>or 3 kinase or an antibody specific for the β or γ-subunits of the G protein, after dissociation of the α-subunit. The G protein that interacts with the cytoplasmic domain is able to activate phosphatidylinositol-3-kinase, and FP is then a fusion of EP with an Src homology 2 or pleckstrin homology domain. Alternatively, MD (in presence or absence of ligand binding) is able to bind one or more adapters, through which EP binds to MD. In particular it includes an enzyme activity (kinase or phosphatase) and then comprises the cytoplasmic part of the epidermal growth factor receptor. Alternatively it activates a separate, receptor-specific enzyme, optionally heterologous to the cell and especially a tyrosine kinase.</p> <p>Preferred Cells: These are prokaryotic or eukaryotic, especially yeast cells lacking a cell wall, particularly on a solid support, especially immobilized on a biochip or enclosed in microchambers. Particularly in (A), in the absence of membrane receptor, at least under certain conditions, a Ras signaling pathway is inactive. Activation of this pathway is temperature dependent, and is associated with a mutation in a cellular GEF or cellular Ras proteins (which are inactive above a particular temperature).</p> <p>WO 200040969-A+/2</p> |
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Preferred Methods: In the method of (1), (A), in which the Ras pathway is not active, is treated with test compound and any activation of the pathway indicates that the test compound is binding to LBD. In particular activation is detected from expression of a reporter gene that requires activation of the pathway or from cellular proliferation. The results may be compared with those for control cells not treated with test compound. In the method of (4), (A) are treated with known ligands in presence or absence of test compounds and any difference in activation of the Ras pathway is detected.

Preferred Kits: These contain (A) and optionally one or more of nucleic acid vectors that express a membrane receptor, EP and/or an adapter. The cells may also include a construct (particularly in a vector) that includes a binding site for a transcription factor (activated by activation of the Ras pathway), a minimal promoter and a reporter gene.

Preferred Compounds: Suitable test compounds are odorants, flavors, peptides and proteins (especially cytokines), growth factors, neurotransmitters, hormones and/or vitamins, also synthetic compounds, particularly derivatives of natural ligands or a toxin such as dioxin. The test compound may be used as a fusion protein, including a potential ligand domain.

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